Clinical Pharmacogenetics

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Medication History: AVOID Mistakes

Allergies? : Is there any medicine that we should not give you for any reason?

Vitamins and Herbs?

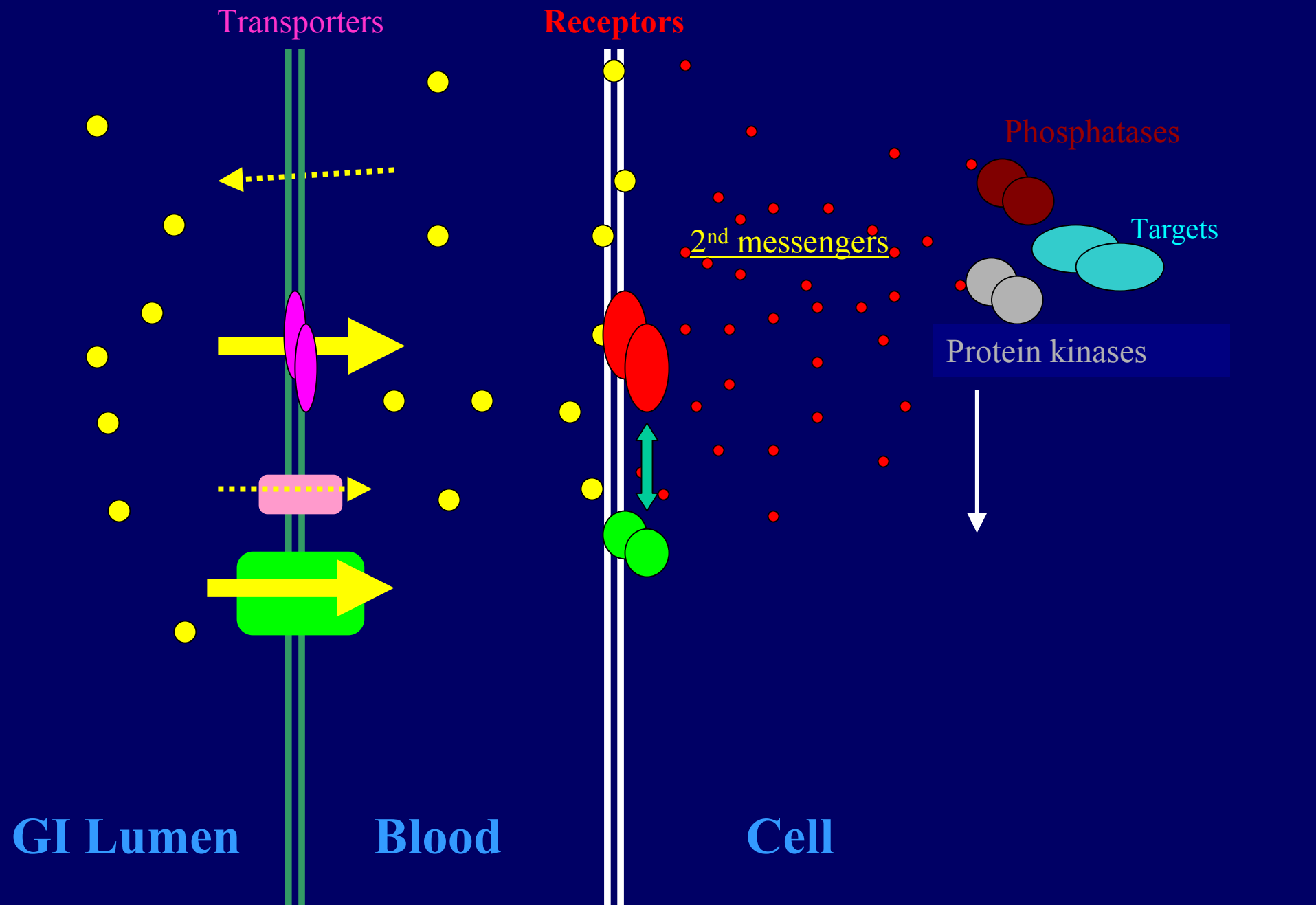
Old drugs? …..as well as current

Interactions?

Dependence?

Mendel: **Family Hx of benefits or problems with any drugs?**

The 20th Century in Small Molecule Pharmacology



Mechanisms of Inherited Genetic Variability

(All are in germ line DNA or mitochondrial DNA)

Single nucleotide polymorphisms (SNPs)

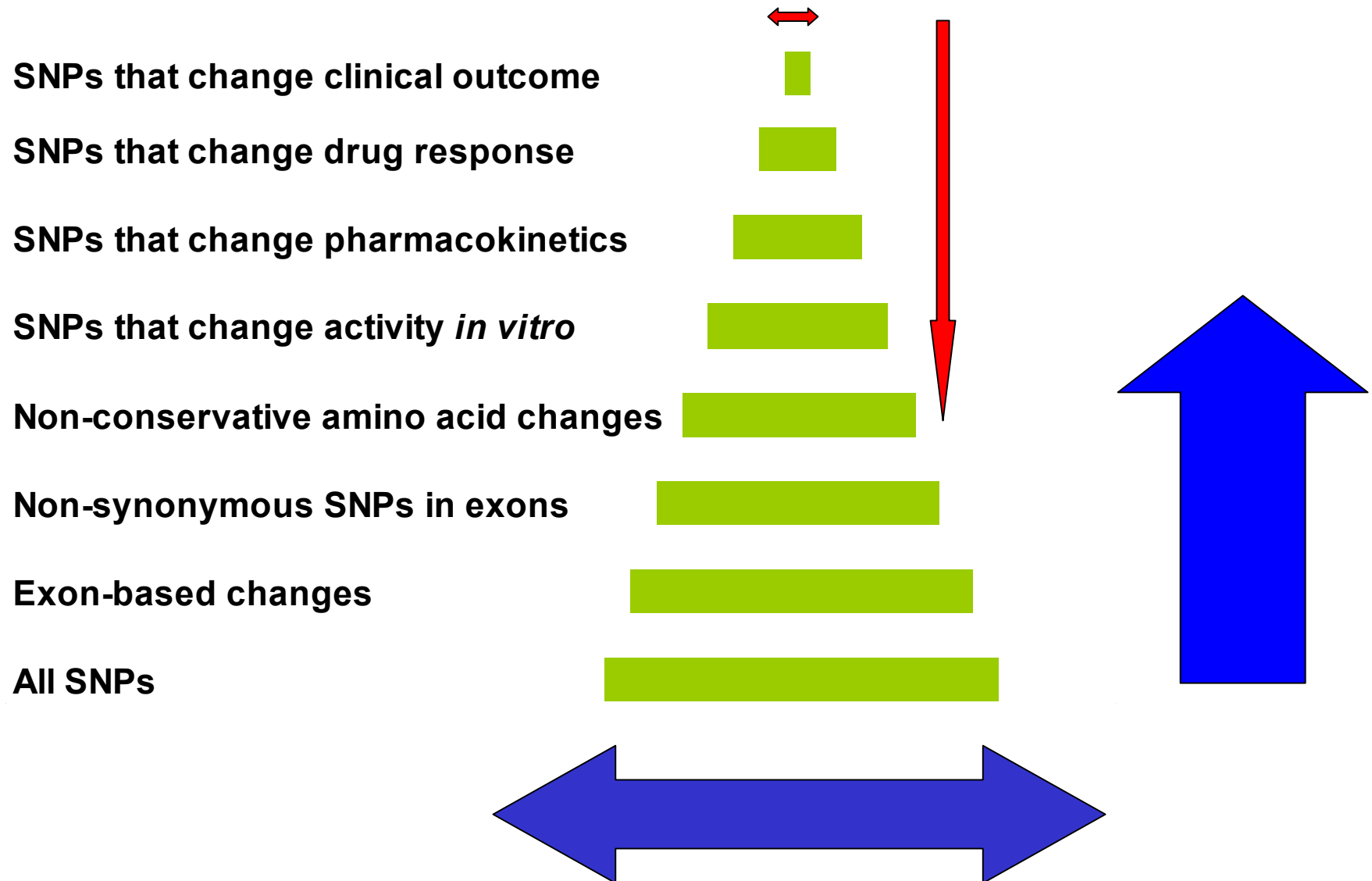
Deletions

Duplications

Insertions

VNTRs

Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)



Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

SNPs that change clinical outcome

SNPs that change drug response

SNPs that change pharmacokinetics

SNPs that change activity *in vitro*

Non-conservative amino acid changes

Non-synonymous SNPs in exons

Exon-based changes

All SNPs

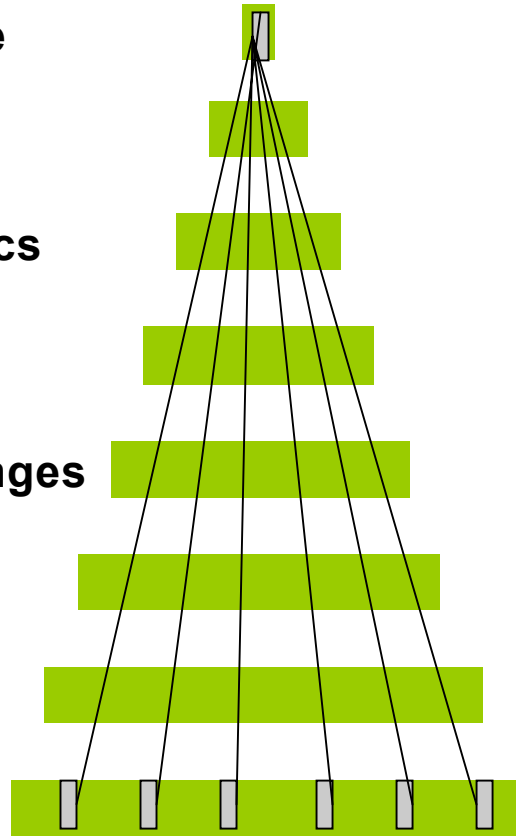


Table 1. The Early History of Pharmacogenetics

- 1932: First Inherited Difference in an Inherited Response to a Chemical: Inability to Taste Phenylthiourea.
- World War II: Hemolysis in African-American soldiers treated with primaquine.
- 1957 “inheritance might explain many individual differences in the efficacy of drugs and in the occurrence of adverse drug reactions” Motulsky.
- 1959: “Pharmacogenetics: the Role of Genetics in Drug Response” . Friedrich Vogel .
- 1959: Genetic influence on isoniazid blood concentrations
- 1964: Genetic variation in ethanol metabolism
- 1977: CYP2D6 polymorphism

Methods in Pharmacogenetics

- Population distribution analysis with Normit plots using a valid probe to detect phenotypic polymorphism (> 1% of population)
- Identification of gene and variants
- Family and twin studies to confirm genetic characteristics (dominant, recessive, Mendelian, maternal etc.)
- Development of a genetic test for DNA variants
- Correlation between genotype and phenotype
- **Application in Clinical Practice**

Phenylthiourea Nontaster Trait

(Snyder *et al*: The inheritance of taste deficiency in man. *Ohio J Sci* 1932: **32**, 436–468.

- 800 families including 2043 children
- Serial Dilution Testing
- Mendelian Inheritance
- US prevalence of the nontaster trait = 30 %.

Genetically Polymorphic Trimethylaminuria

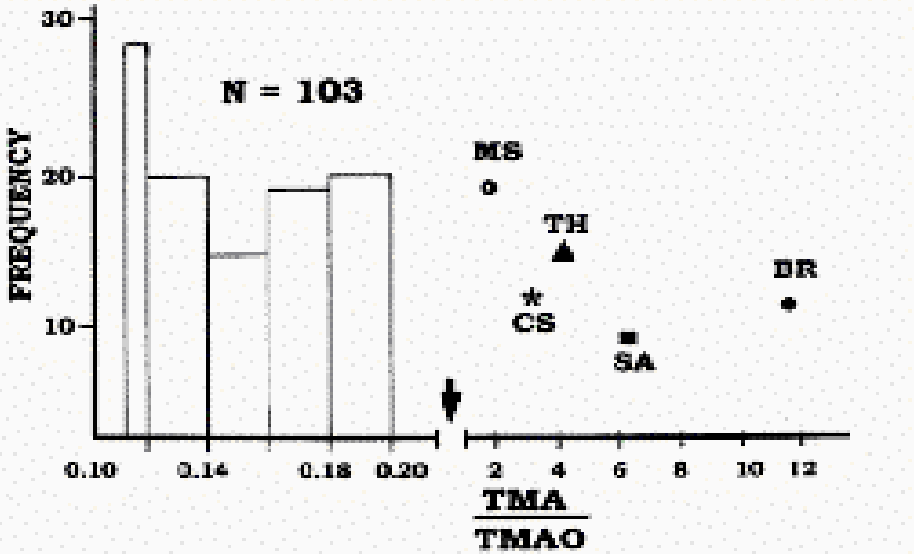


Fig. 1. Population frequency of the urinary TMA:TMAO ratios (μmol per 24 h) for 103 normal healthy volunteers. The ratio of each affected proband is also shown for comparison, with the position on the *y*-axis arbitrarily assigned.

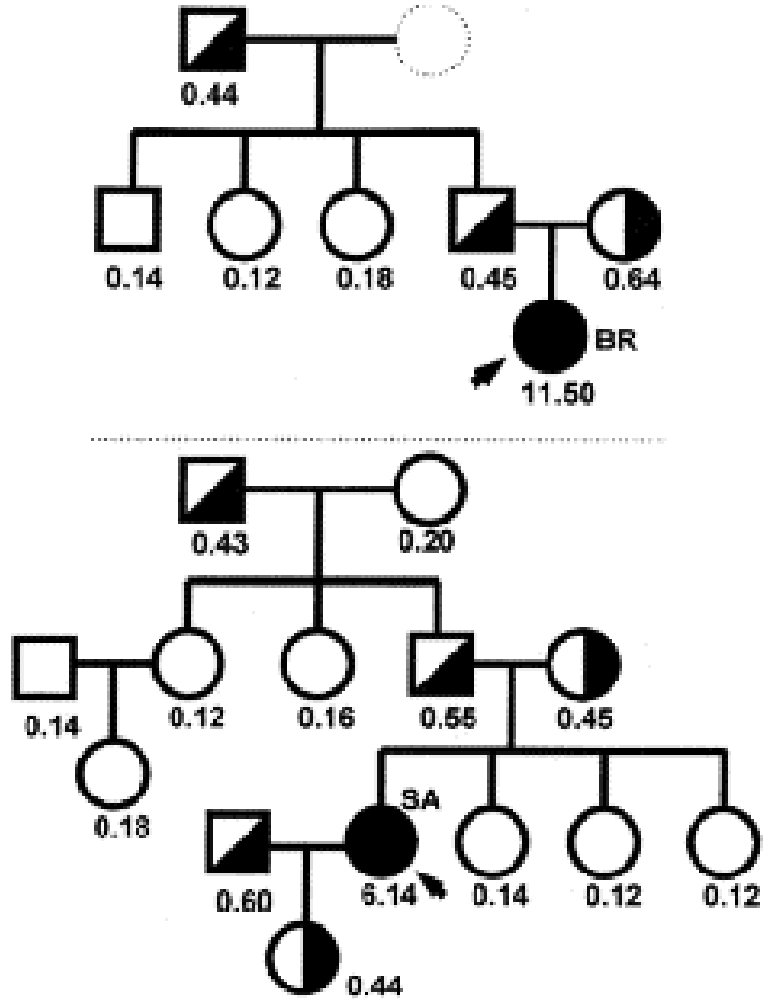


Fig. 2. Family studies on the urinary excretion (μmol per 24 h) of TMA and TMAO under normal dietary conditions. All values shown are the TMA/TMAO ratios. Each affected proband is indicated by an arrowhead.

From: **Thithapandha, A.**
A pharmacogenetic study of trimethylaminuria in orientals
Pharmacogenetics (1997) **7**, 497–501

Table 2: Properties of an ideal pharmacogenetic probe to measure phenotype

- Specific for the trait in question
- Sensitive
- Simple to carry out
- Inexpensive
- Easy to assay
- Clinically benign

Examples of Genetic Effects on Human Drug Absorption, Action and Elimination

- Absorption:
- Alcohol Dehydrogenase
  - Aldehyde Dehydrogenase
  - Cytochrome P450 3A5
  - P-glycoprotein
  - **Multidrug Resistance Transporter (MRP)**

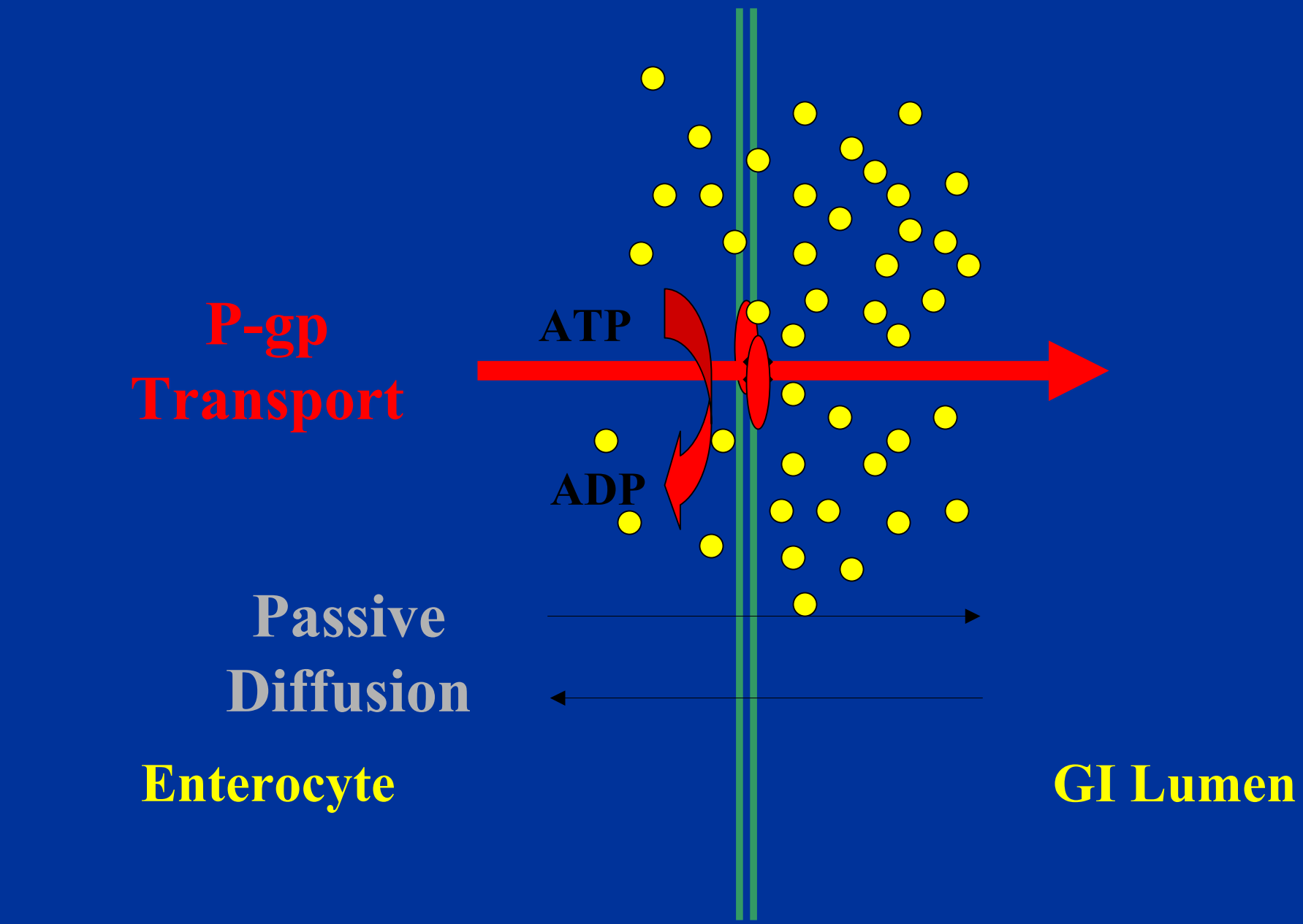
- Action
  - G-protein variants
  - Angiotensin II receptor and Angiotensinogen variants
  - β_2 receptor
  - Dopamine D4 receptor
  - Endothelial NO synthase
  - $5HT_4$ receptor

Examples of Genetic Effects on Human Drug Absorption, Action and Elimination (continued)

- Cytochrome P450 2A6
- Cytochrome P450 2C9
- Cytochrome P450 2C19
- Cytochrome P450 2D6
- Cytochrome P450 3A5
- Regulation of cytochrome P450 3A4
- Dihydropyridine Dehydrogenase (DPD)
- UDP-Glucuronyl Transferase 1A1 (UGT 1A1)
- Glutathione - S - Transferase (GST)
- Thiopurine methyl transferase (TPMT)
- Flavin Mono-Oxygenase 3 (FMO-3)

Genetics and Drug Absorption

Digoxin Transport across the GI lumen



P-Glycoprotein Pharmacogenetics :

Effect of a “wobble” (no coding change) SNP in exon 26

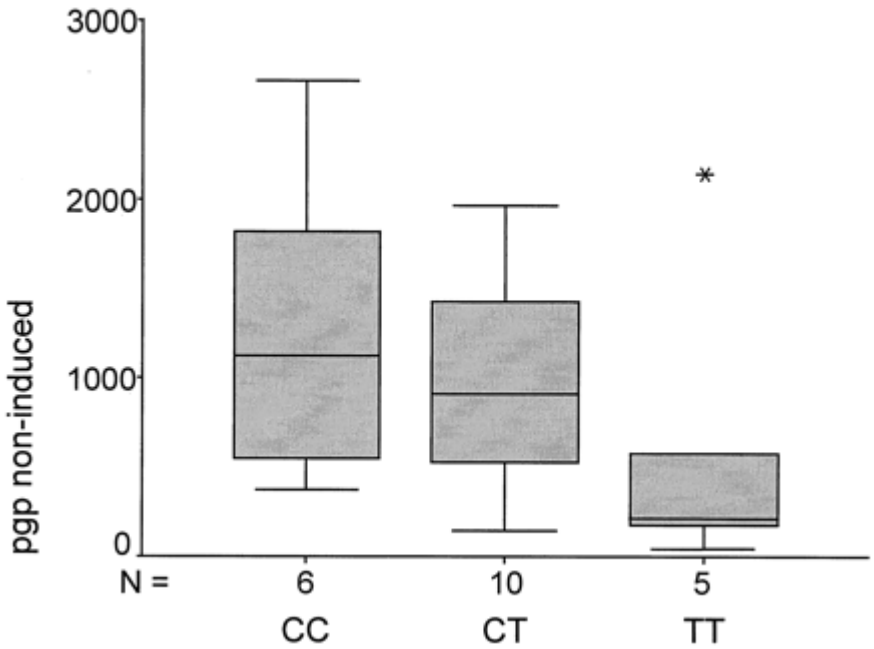
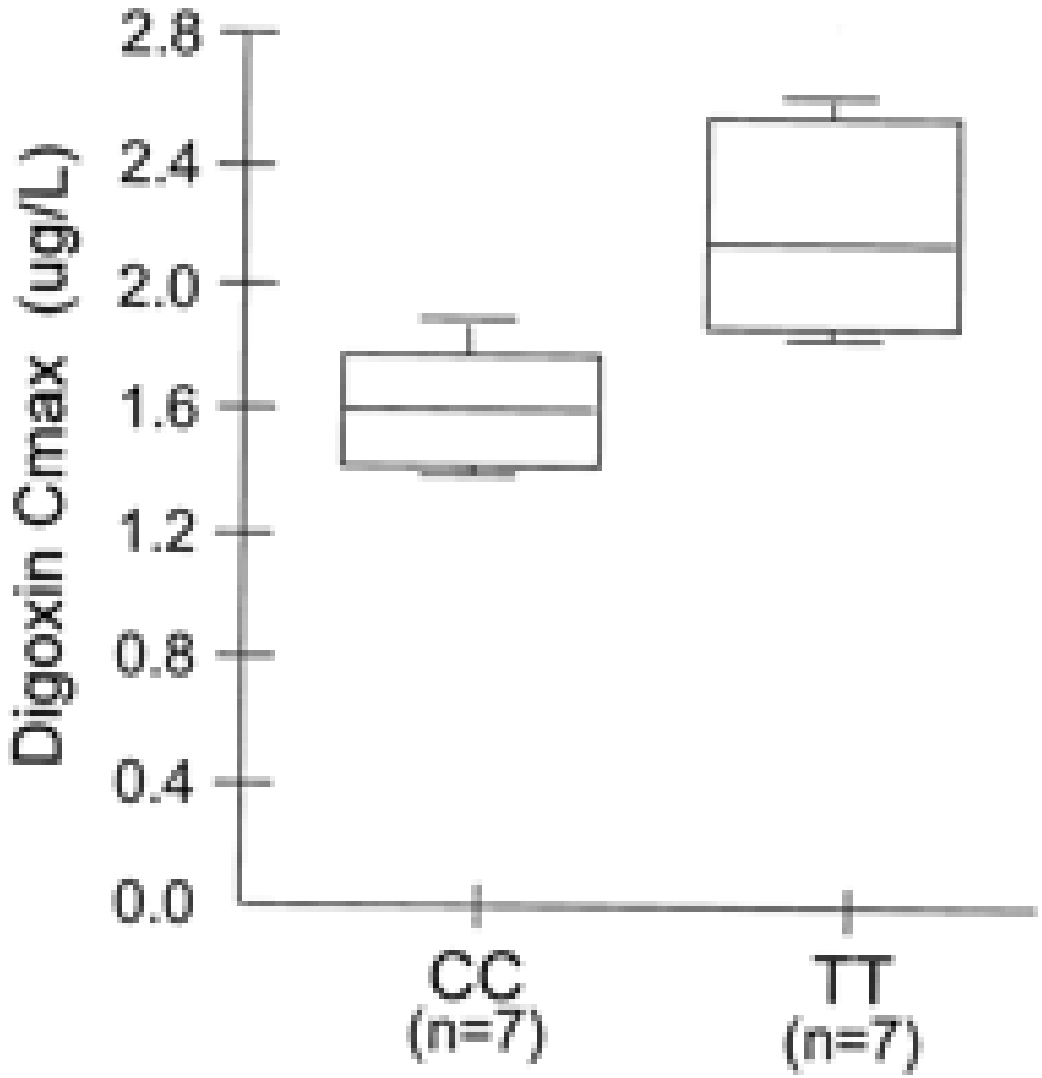


Fig. 3. Correlation of the exon 26 SNP with MDR-1 expression. The MDR-phenotype (expression and activity) of 21 volunteers and patients was determined by Western blot analyses. The box plot shows the distribution of MDR-1 expression clustered according to the MDR-1 genotype at the relevant exon 26 SNP. The genotype-phenotype correlation has a significance of P = 0.056 (n = 21).

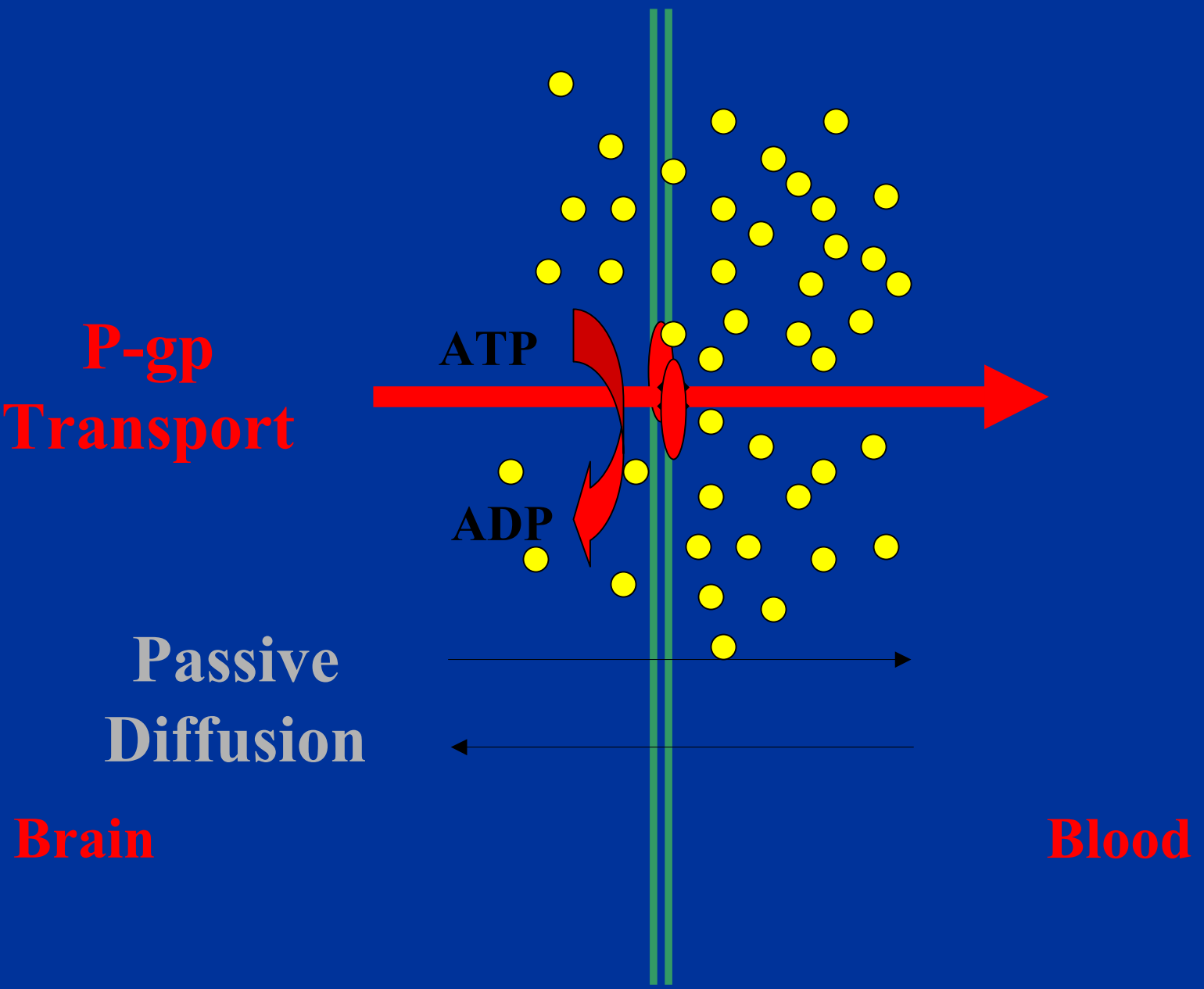
Eichelbaum et al. Proc Nat Acad Sci March, 2000.

0.25 mg of digoxin po at steady state



Eichelbaum et al, Proc Nat Acad Sci, 2000:March

Digoxin Transport across the Blood-Brain Barrier



Note

- Pharmacokinetic changes do not always have predictable pharmacodynamic consequences
- Wobble changes may be important even though the mechanism involved is unclear

Aldehyde Dehydrogenase Genetics

- 10 human ALDH genes
- 13 different alleles
- autosomal dominant trait because of lack of catalytic activity if ***one subunit*** of the tetramer is inactive
- ALDH2 deficiency results in build up of toxic acetaldehyde
- Absent in up to 45% of Chinese, not at all in Caucasians or Africans

Effect of Alcohol Dehydrogenase 3 genotype on Risk of cardiovascular Disease

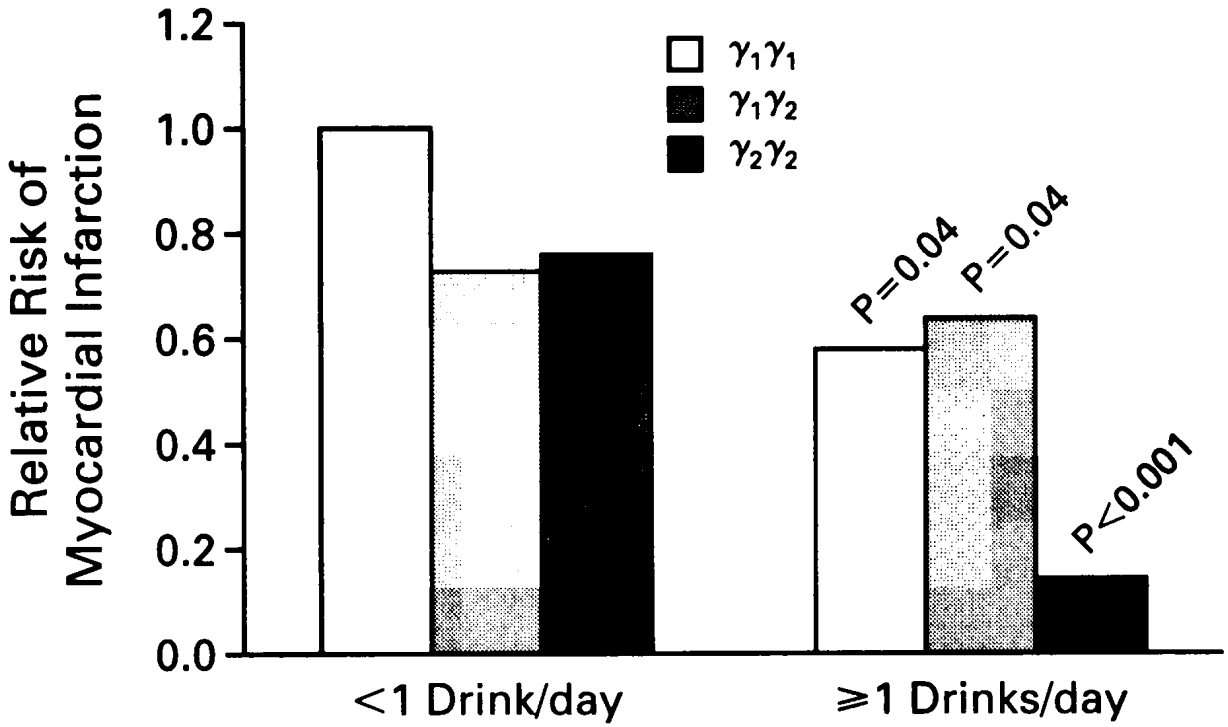


Figure 1. Multivariate Relative Risk of Myocardial Infarction According to the *ADH3* Genotype and the Level of Daily Alcohol Consumption.

Hines et al: N. Engl. J.Med 344;549-555,2001

Physician's Health Study:

Nurses Health Study:

325 women not on HRT

Hines et al: N. Engl. J.Med 344;549-555,2001

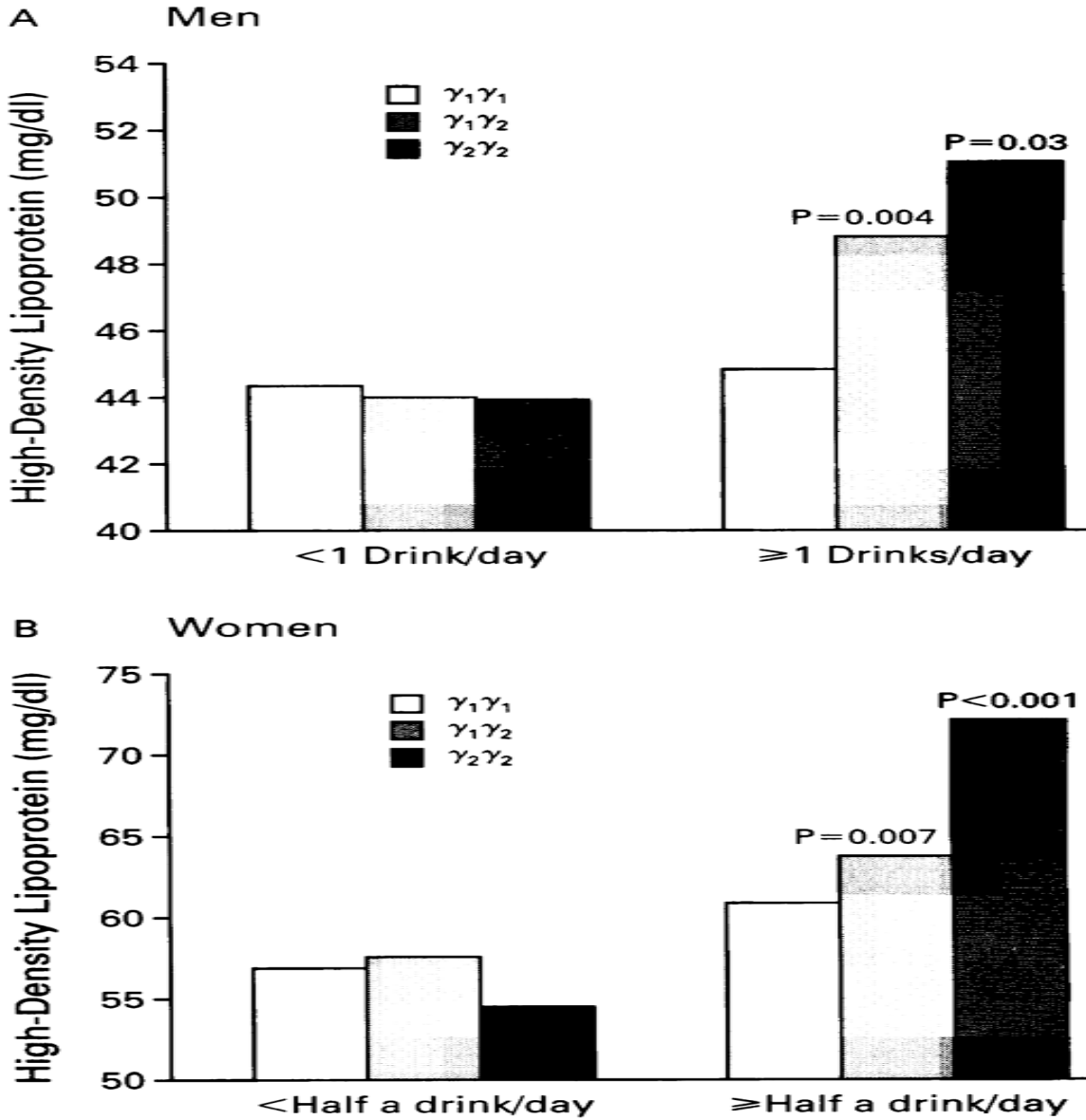
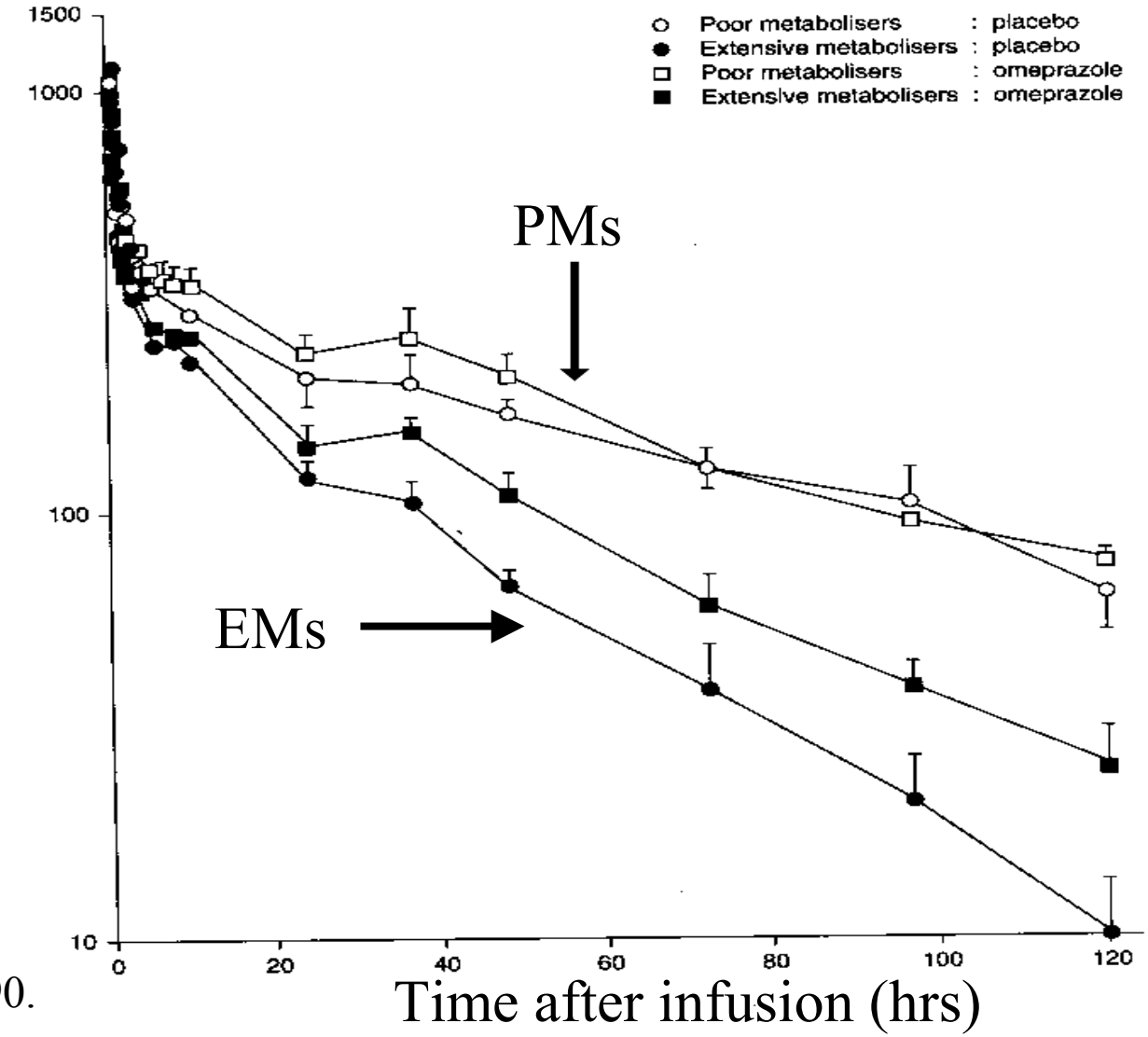


Figure 2. Adjusted High-Density Lipoprotein Levels Accord to the Level of Alcohol Consumption and the *ADH3* Genot in 385 Patients with Myocardial Infarction and 385 Control the Physicians' Health Study (Panel A) and 325 Postmenopau Women in the Nurses' Health Study Who Were Not Receiv Hormone-Replacement Therapy (Panel B).

Genetics and Drug Elimination

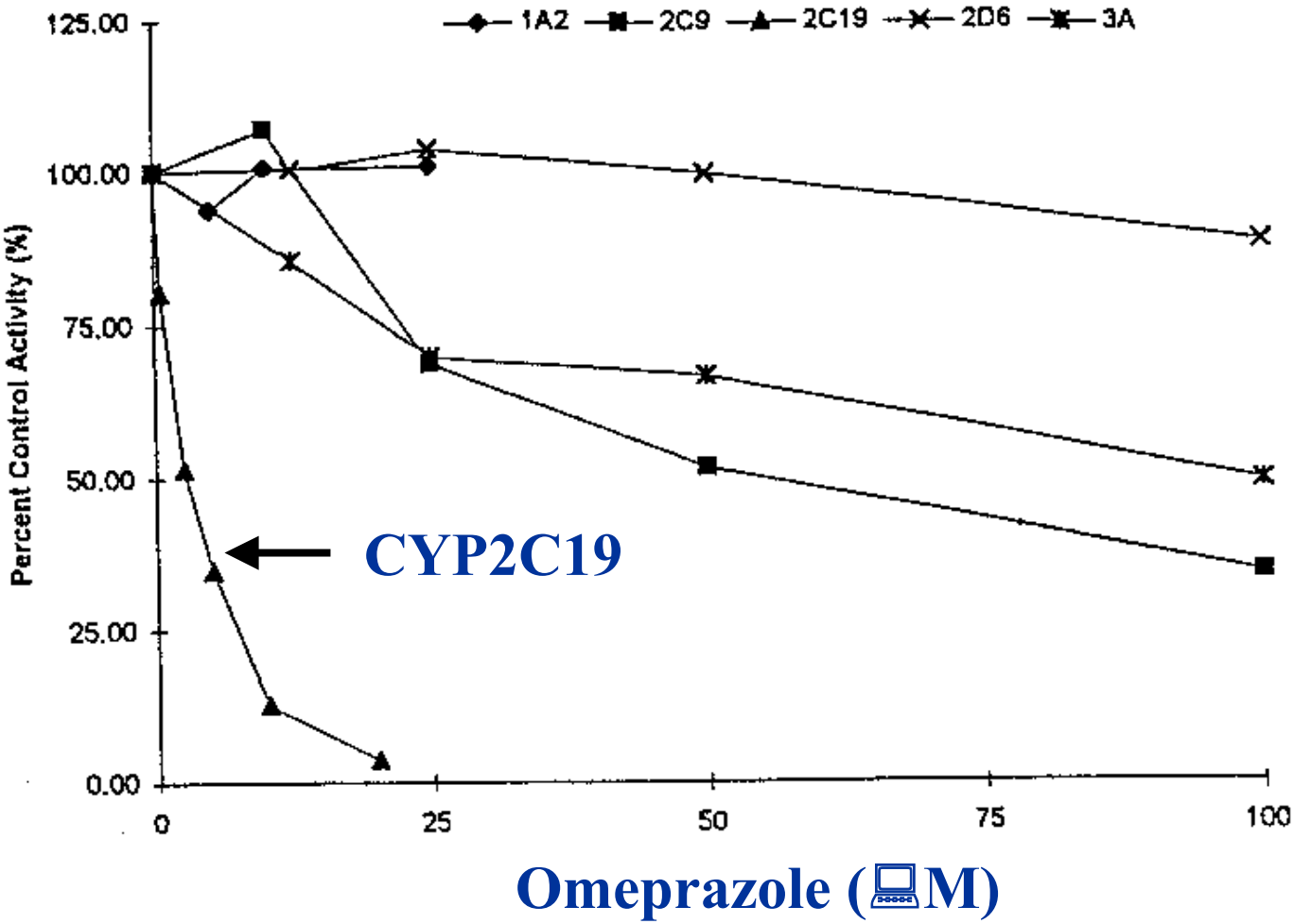
Effect of *CYP2C19* genotype and omeprazole on diazepam pharmacokinetics



[Diazepam] (nM)

Andersson *et al*, 1990.

Specific CYP2C19 inhibition by omeprazole



Ko JW and Flockhart DA, 1997.

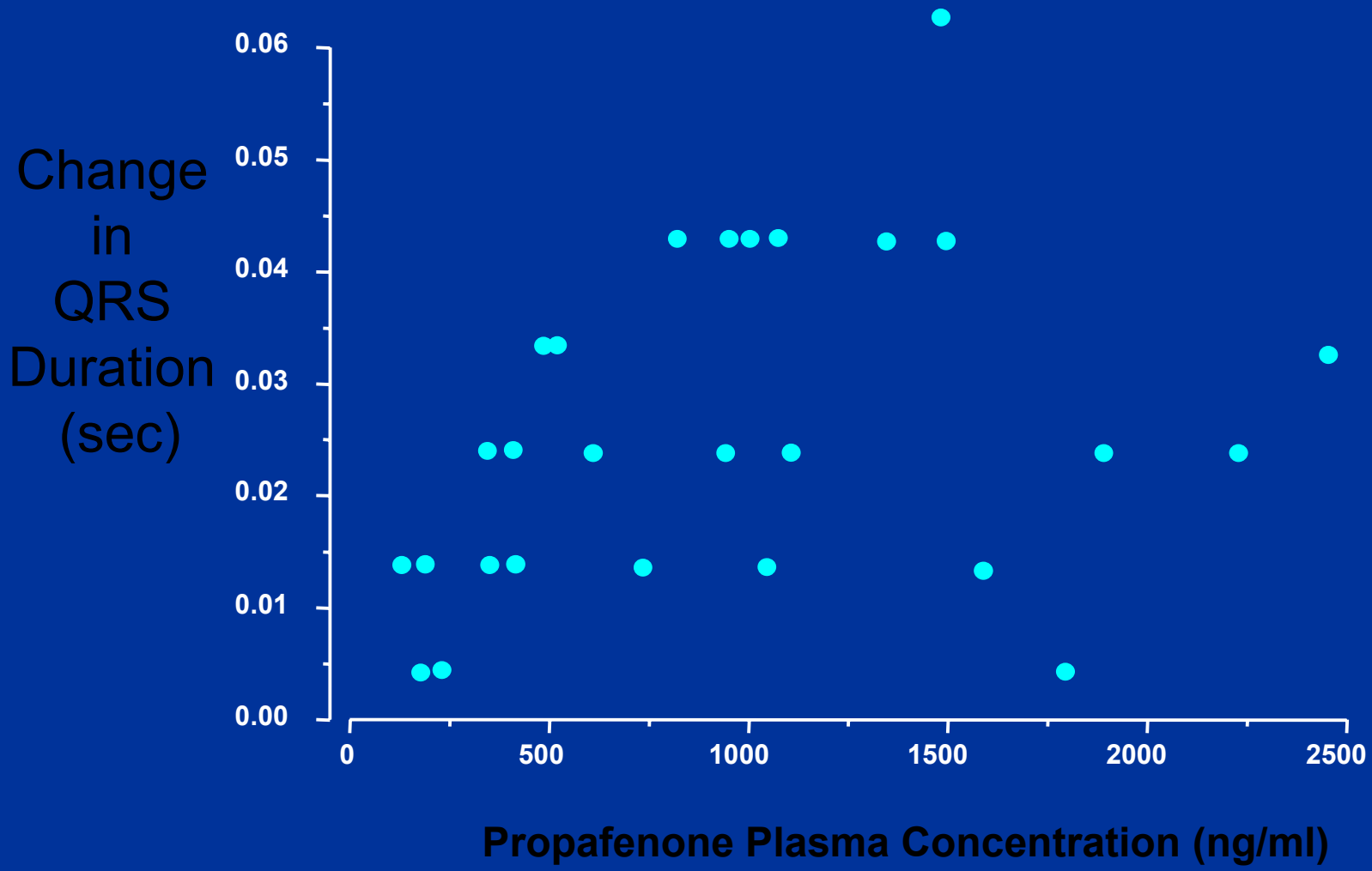
Lessons Learned

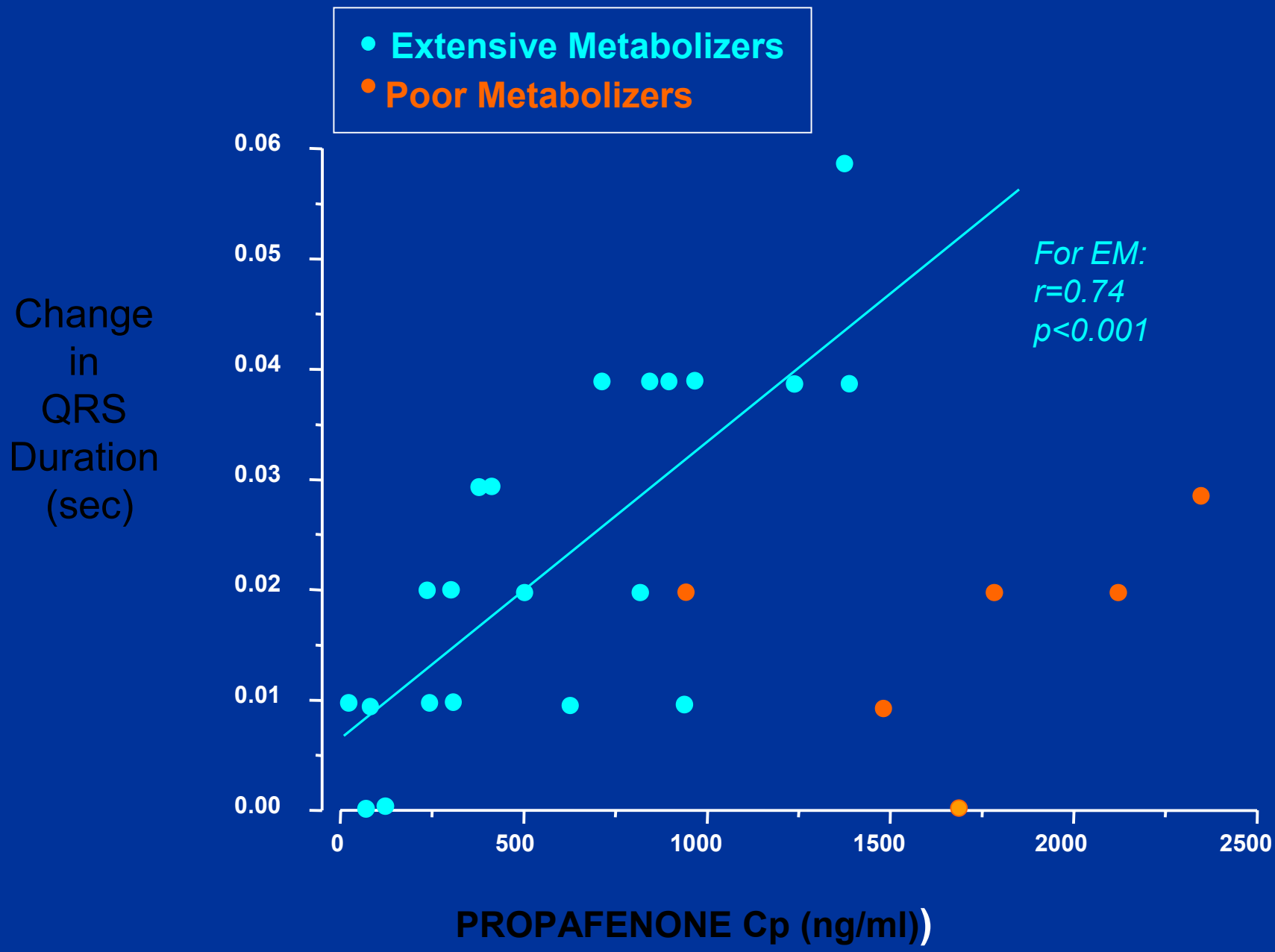
- The environment can mimick genetic effects convincingly: tests of phenotype will always be important
- Genetics is not everything, so every genetic association must be examined for potential environmental confounders

Cytochrome P450 2D6

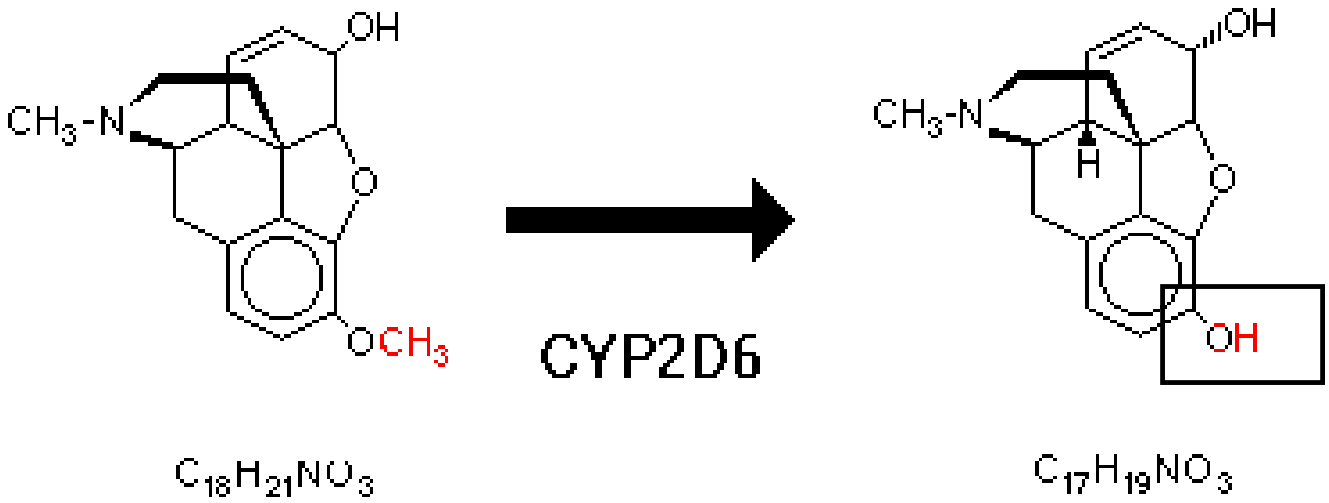
- Absent in 7% of Caucasians
- Hyperactive in up to 30% of East Africans
- Catalyzes primary metabolism of:
  - propafenone
  - codeine
  - β-blockers
  - tricyclic antidepressants
- Inhibited by:
  - fluoxetine
  - haloperidol
  - paroxetine
  - quinidine

Propafenone




Extensive Metabolizers
Poor Metabolizers
Change in QRS Duration (sec)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
500
1000
1500
2000
2500
PROPAFENONE Cp (ng/ml))
For EM:
r=0.74
p<0.001

The O-dealkylation of codeine by CYP2D6



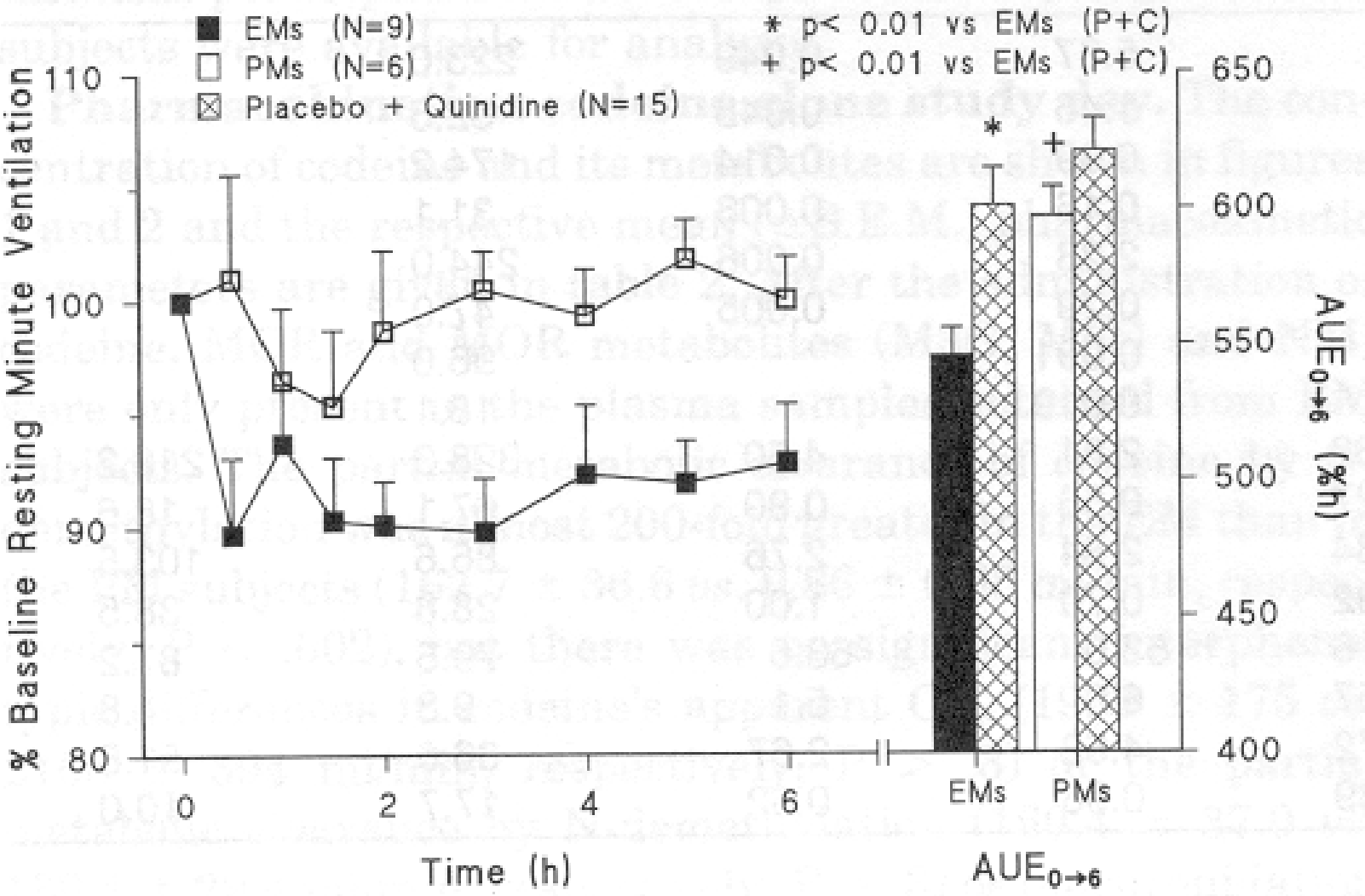
$C_{18}H_{21}NO_3$

$C_{17}H_{19}NO_3$

codeine

morphine

Effect of CYP 2D6 Genotype on the Efficacy of Codeine



From: Caraco et al: JPET;1996:278:1165-1174.

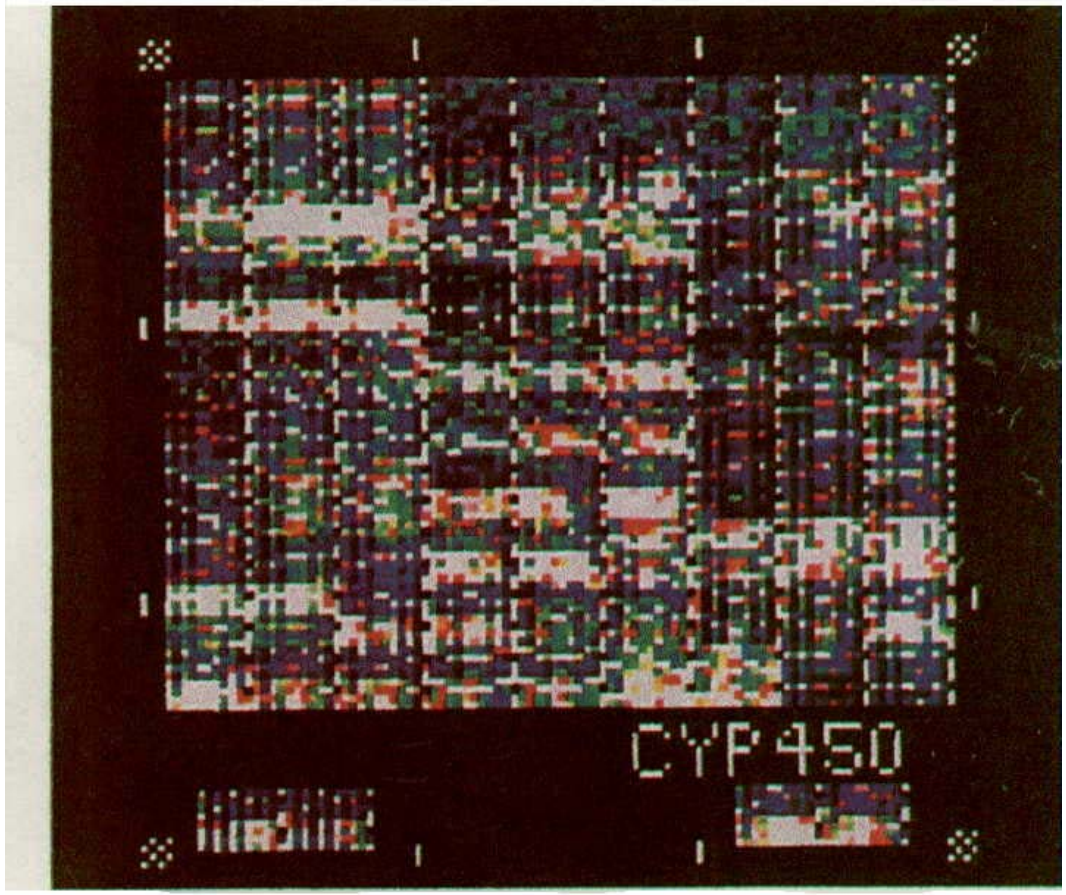
CYP2D6 Alleles

- 41 as of May, 2001
- 24 alleles have no activity
- 6 have decreased activity
- The *2 variant can have 1,2,3,4,5 or 13 copies i.e increased activity

Chip Determination of CYP genotype

- Mutations Detected
  - *CYP2D6* *1A, *1B, *2, *2XN, *3, *4A, *4B, *4C,*4D, *4E, *5, *6A, *6B, *7, *8, *9, *10A, *10B, *11
  - CYP2C19 *1, *2, *3

Oligonucleotide array for cytochrome P450 genotesting



From: Flockhart DA and Webb DJ. *Lancet* End of Year Review for Clinical Pharmacology, 1998.

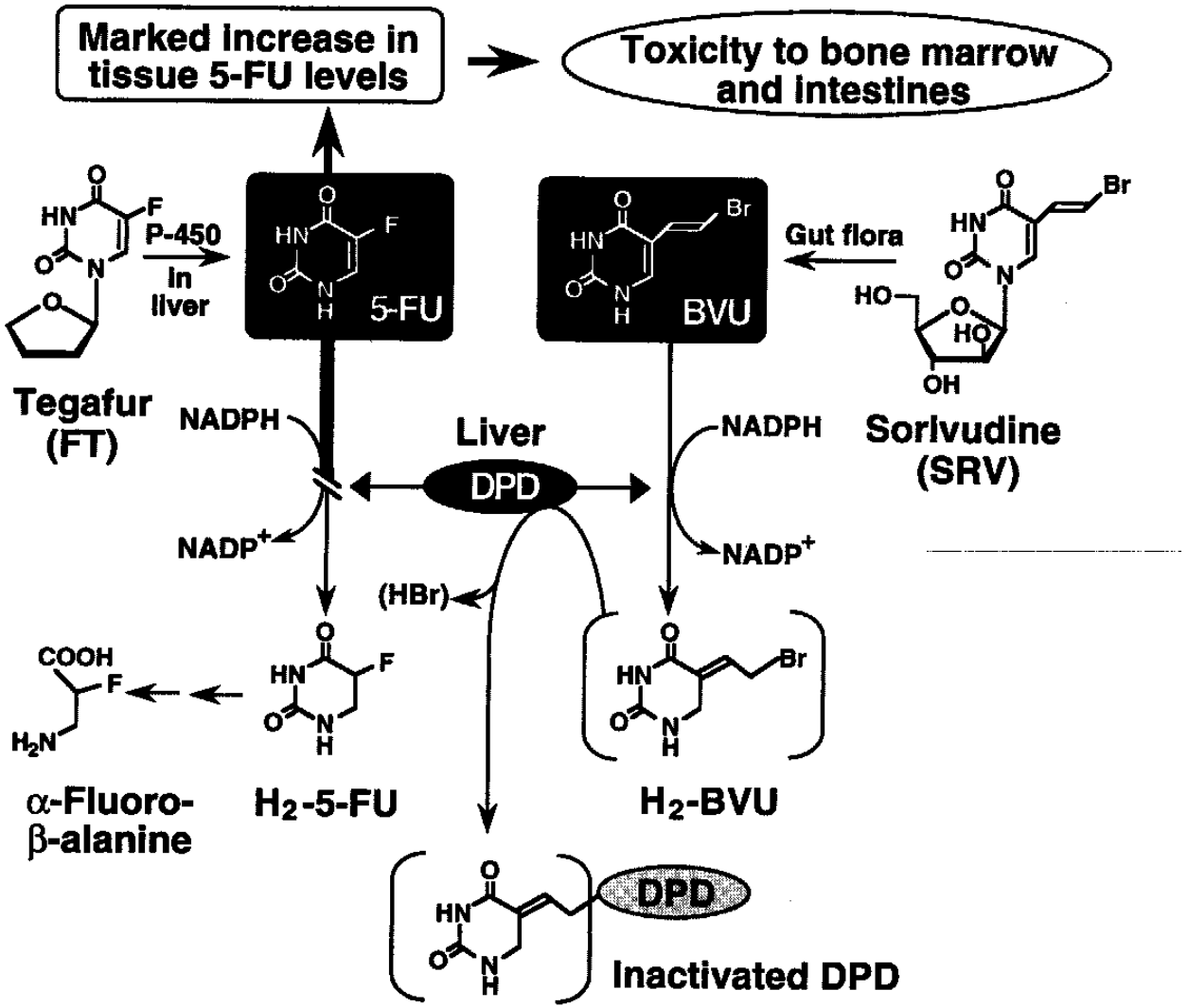
Lessons from CYP Pharmacogenetics

- Multiple genetic tests of one gene may be needed to accurately predict phenotype
- Genetic tests need validation just as any others do

Dihydropyridine Dehydrogenase

- Absent in ~ 3% of Caucasians
- Responsible for metabolism of 5-fluorouracil
- 80-90% of 5-FU is metabolized, 10 - 20% is renal
- Deficient patients treated with conventional doses of 5-FU experience diarrhea, stomatitis, mucositis, myelosuppression and neurotoxicity.

Dihydropyridine Dehydrogenase



Okuda et al. Eighteen deaths due to an interaction with DPD.JPET 1998;287:791-809

Genetic alterations in Phase 2 enzymes with clinical consequences

UDP Glucuronyl Transferase 1A1

- Responsible for Gilbert's Bilirubinemia
- absent in ~15% of Caucasians
- < 5% Asians
- > 50% of Africans
- > 50% of Hispanics
- Decreased activity in hypoglycemic and malnourished conditions, so Gilbert's hyperbilirubinemia is "revealed" by these conditions.

N-Acetylation Polymorphism NAT-2

- Late 1940's : Peripheral Neuropathy noted in patients treated for tuberculosis.
- 1959 : Genetic factors influencing isoniazid blood levels in humans. *Trans Conf Chemother Tuberc* 1959: **8**, 52–56.

NAT-2 substrates
(All have been used as probes)

- Caffeine
- Dapsone
- Hydralazine
- Isoniazid
- Procainamide

Incidence of the Slow Acetylator NAT-2 phenotype

- 50% among Caucasians
- 50% among Africans
- 20% among Egyptians
- 15% among Chinese
- 10% among Japanese

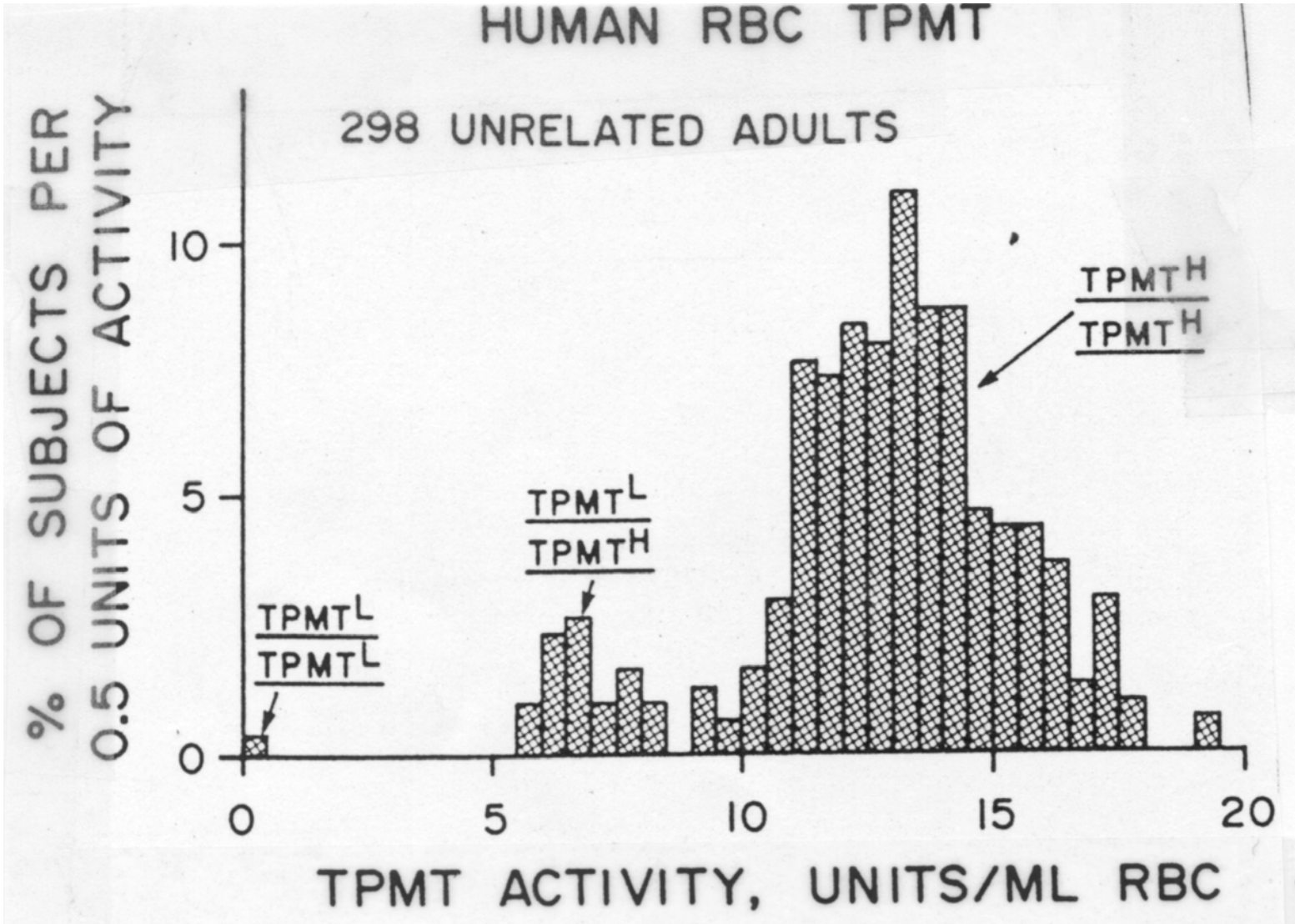
Clinical relevance of the NAT-2 polymorphism

- Higher isoniazid levels, greater neuropathy in slow acetylators
- Faster ANA appearance with procainamide in slow acetylators
- Hydralazine-induced *lupus erythematosus* is much less common in rapid than slow acetylators

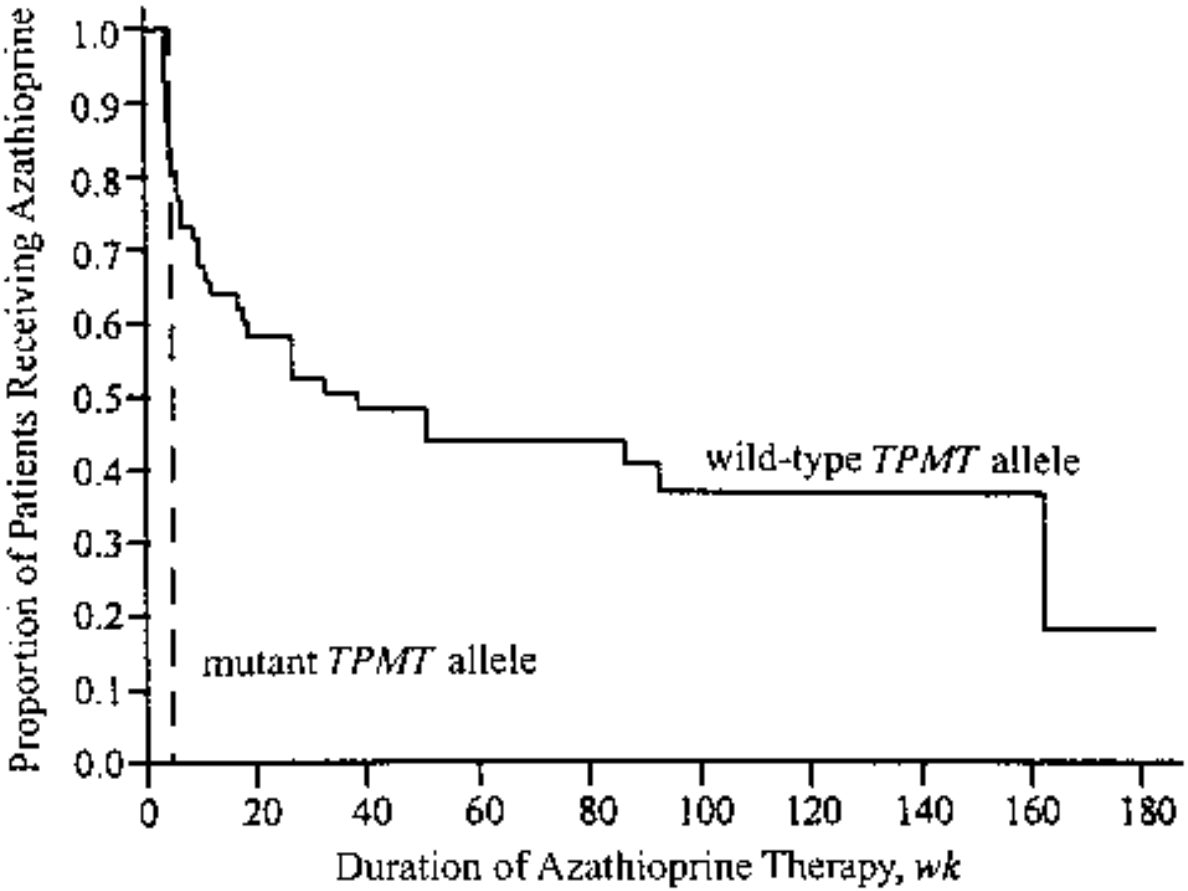
Thiopurine Methyl Transferase

- Homozygous mutants are 0.2% of Caucasian Populations
- Heterozygotes are ~ 10%
- Homozygous wild type is 90%
  - Metabolism of Azathioprine
  - 6-Mercaptopurine

Thiopurine Methyl Transferase Deficiency



Effect of TPMT genotype on duration of Azathioprine therapy.



From: Macleod et al: Ann Int Med 1998;

Examples of Human Receptors shown to be genetically polymorphic with *possible* alterations in clinical phenotype

- G-proteins
- Angiotensin II receptor and angiotensinogen
- Angiotensin converting enzyme
- β_2 receptor
- Dopamine D_4 receptor
- Endothelial NO synthase
- $5HT_4$ receptor

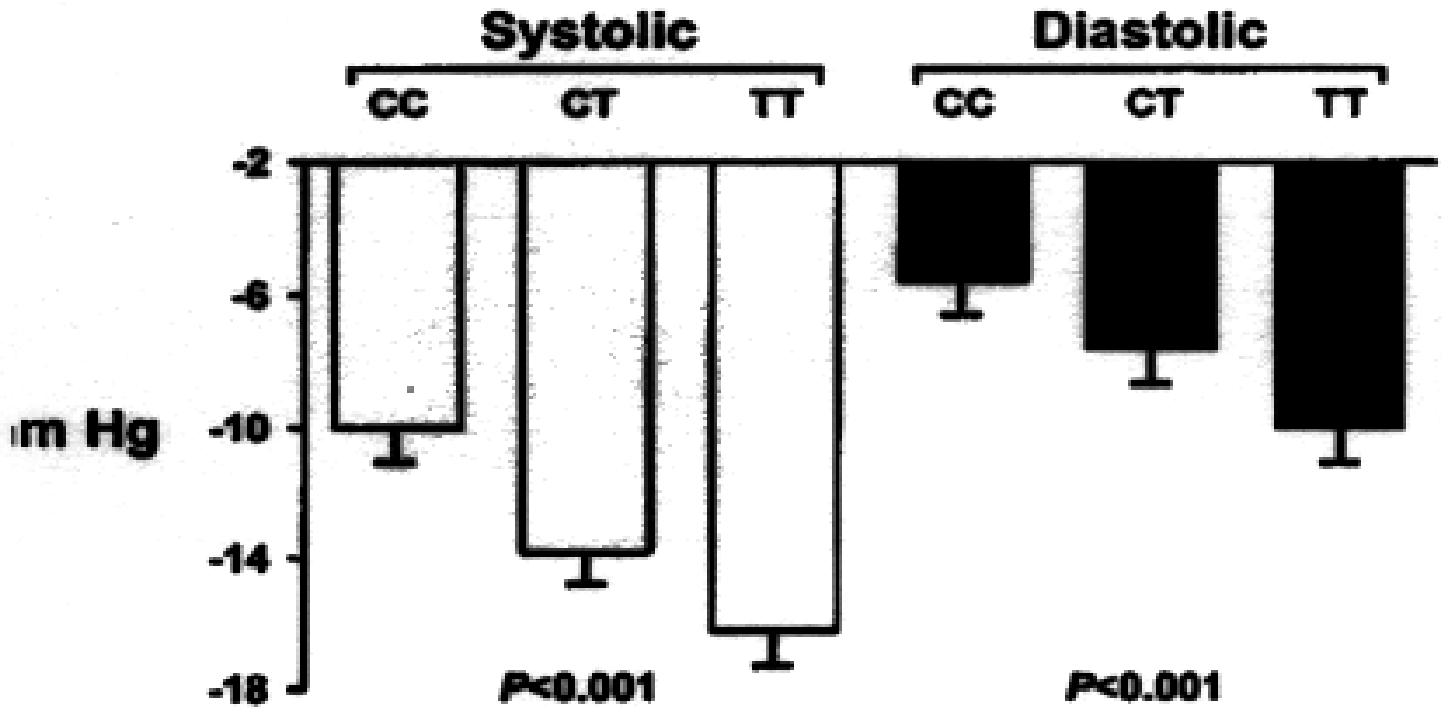
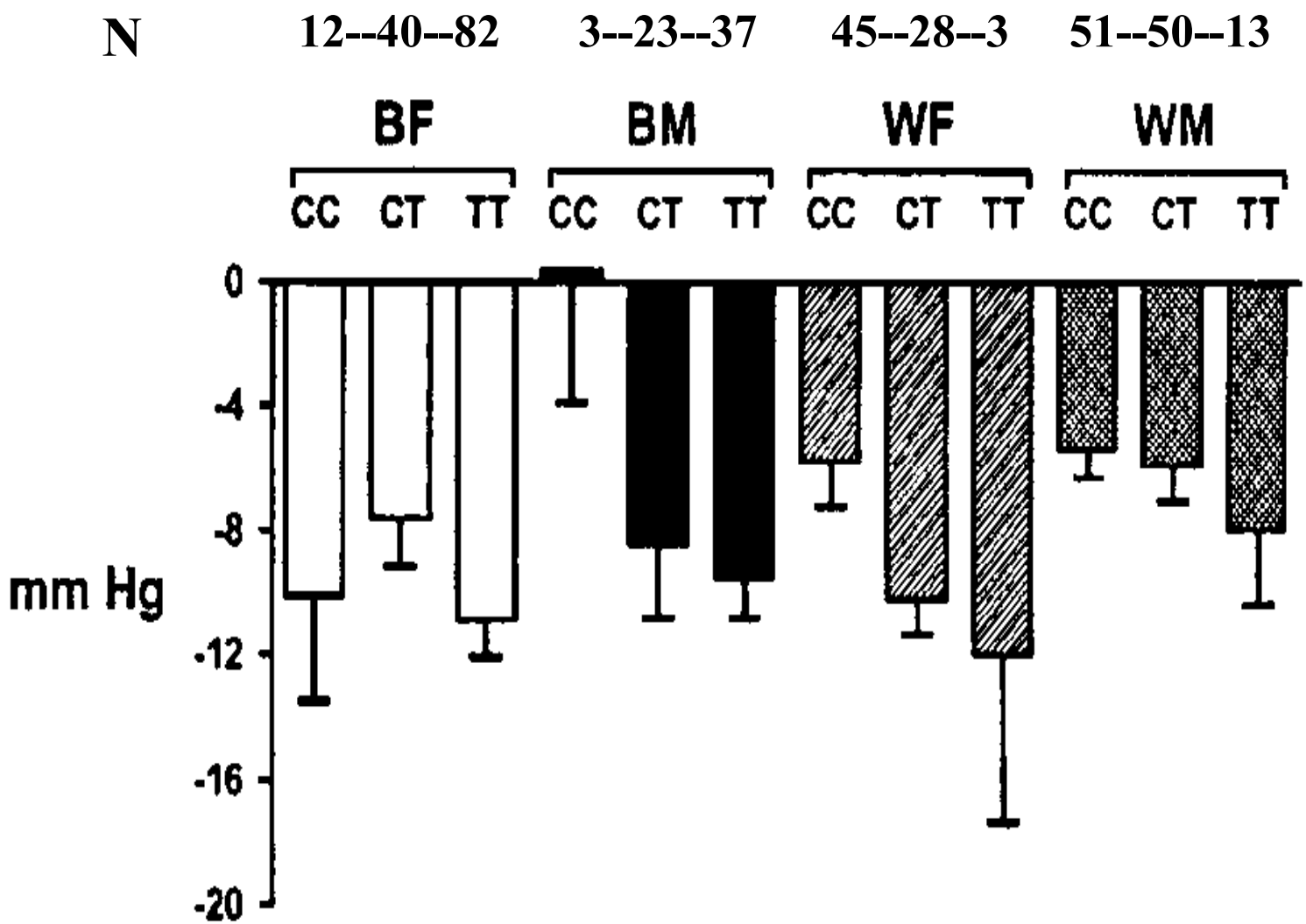
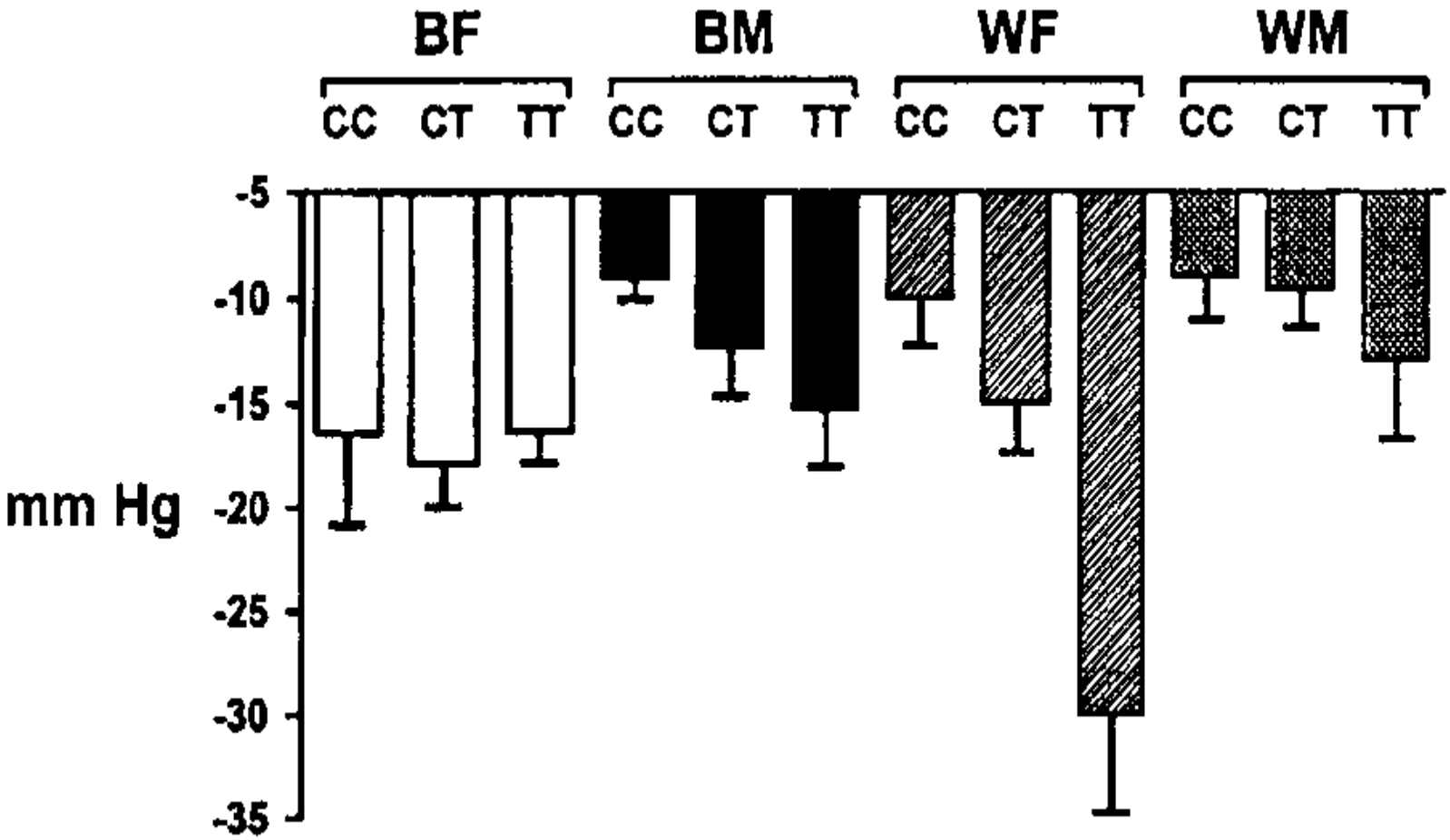


Figure 1. Genotype-specific systolic and diastolic pressure responses to hydrochlorothiazide therapy in the pooled sample of 197 blacks (134 men, 63 women) and 190 non-Hispanic whites (76 men, 114 women) with previously diagnosed essential hypertension. The C825T genotype groups are denoted CC, CT, and TT.



Genotype-specific **Diastolic** BP Responses to 25 mg HCTZ for 4 weeks

N



Genotype-specific **Systolic** BP Responses to 25 mg HCTZ for 4 weeks

Missense E298D Variant of Endothelial NO Synthase in Humans

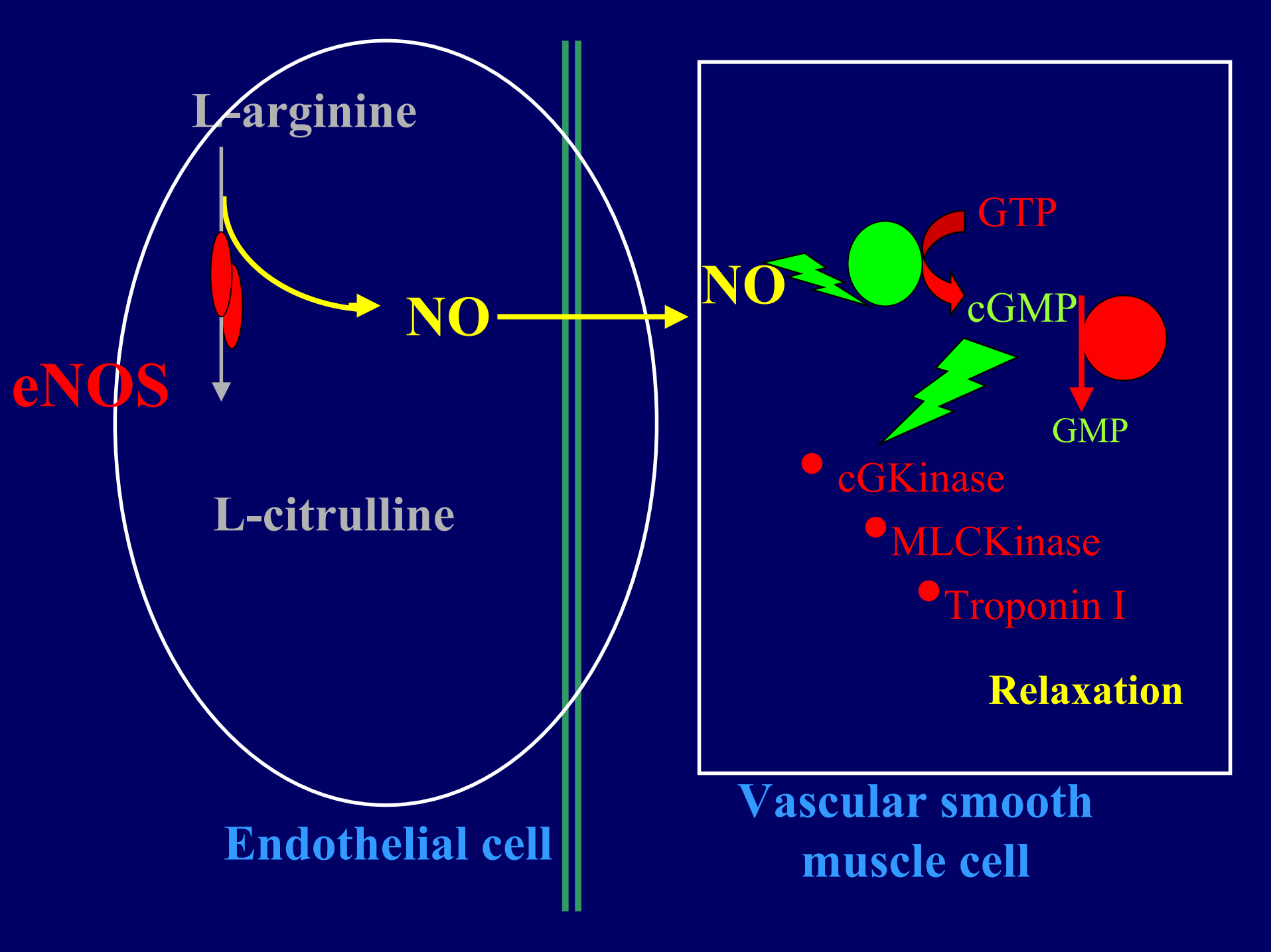
- **A single nucleotide polymorphism**

  G894→T leading to E(Glu)298→D(Asp)

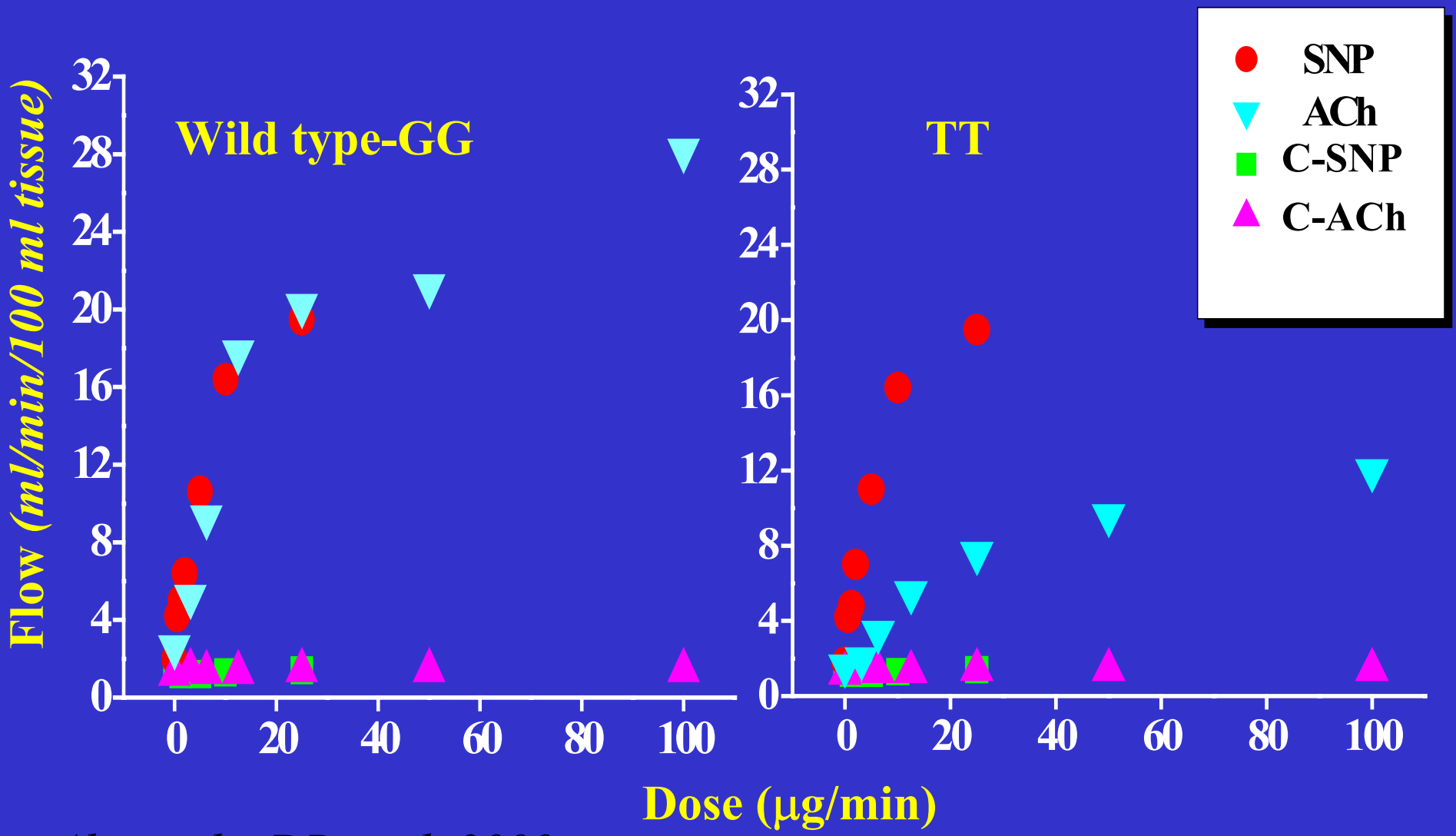
  in exon 7 of human eNOS cDNA.

  (Yoshimura M. et al., Hum Genet 1998, 103:65-69).

- **More frequent in patients with various cardiovascular diseases.**
- **However, no study has demonstrated a physiological/ functional change related to the mutation.**


L-arginine
eNOS
NO
L-citrulline
Endothelial cell
NO
GTP
cGMP
GMP
cGKinase
MLCKinase
Troponin I
Relaxation
Vascular smooth muscle cell

Forearm Blood Flow in Response to Drug Infusion



Abernethy DR et al. 2000

Current Methods for genetic testing

- By phenotype: metabolic probe drug or Western blot
- By PCR with mutation-specific endonuclease
- By PCR and allele-specific hybrization
- By oligonucleotide chip hybridization
- By laser lithography - guided oligonucleotide chip hybridization.
- By rapid throughput sequencing

Estimated cost to the patient of Genetic Tests in Clinical Practice

- By simple PCR for one mutation: ~$10
- For 50 mutations: ~$150
- By Chip for ~ 20 mutations: ~ $70
- By Chip for 100 mutations: ~ $250

Ethical and legal issues within pharmacogenetics

- Risk of Loss of Patient Confidentiality
  - Need for anonymized DNA storage systems
- Risk that existing patents will stifle progress
  - Need for careful interpration of Bayh-Dole
- Untangling the relationship between genetics and self-described ethnicity

Clinical Pharmacogenetics Summary

- A good phenotyping probe is critical
- Genetic tests need validation just as any other tests
- A potent inhibitor can mimick a genetic polymorphism
- Not all genetic polymorphisms have a phenotypic correlate, or clinical effect
- The clinical relevance of genetic polymorphisms is greatest with drugs of narrow therapeutic range, *but not confined to them*
- The cost of genetic testing is not likely to be limiting

Pharmacogenetics Websites

- The SNP consortium: http://brie2.cshl.org
- The Human Genome:
  www.ncbi.nlm.nih.gov/genome/guide/H_sapiens.html
- CYP alleles: www.imm.ki.se/CYPalleles/
- Drug Interactions: www.drug-interactions.com